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Effects of the glucagon-like peptide-1 (GLP-1) analogues exenatide, exenatide extended-release, and of the dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin on glucose metabolism in healthy cats

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Effects of the glucagon-like peptide-1 (GLP-1) analogues exenatide, exenatide extended-release, and of the dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin on glucose metabolism in healthy cats

Incretin analogues and inhibitors of the breakdown of endogenous incretins are novel classes of antidiabetic drugs that increase glucose-stimulated insulin secretion in rodents and humans. Objectives were to test whether the GLP-1 analogues exenatide (*E*), exenatide extended-release (*E e-r*) and the DPP-4 inhibitor sitagliptin (*S*) can be safely used in cats, to identify the most effective drug and to test the effects of prolonged administration of *E e-r*. *E* was given to three cats at 0.2-2µg/kg, q12h, subcutaneously, for 5 days. *E e-r* was given to three cats at 40-400µg/kg, subcutaneously, once. *S* was given to three cats at 1-10mg/kg, q24h, orally, for 5 days. Five days after initiation of treatment, meal response tests (MRT) were performed and glucose, insulin and glucagon areas under the curve (AUC) calculated. Prolonged *E e-r* treatment was tested in three cats each at 100 and 200µg/kg, q7d, for 5 weeks, followed by MRT. Compared to treatment-naïve cats, *E* increased insulin AUC by 224%, 258%, 331% and 93%, *E e-r* by 127, 169, 178 and 95%, and *S* by 32%, 69%, 62%, and 43%, respectively. Glucose and glucagon AUC were unaffected by drug or dose. *E e-r* at 200µg/kg induced a more pronounced enhancement of insulin secretion than at 100µg/kg. The three tested drugs are safe to use in cats and enhance insulin secretion. Incretin-based therapy may represent a novel treatment option for management of diabetic cats.

Keywords: Incretins, cats, diabetes mellitus, exenatide, sitagliptin

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Inkretin-Analoga und Inhibitoren des Abbaus endogener Inkretine sind neue Klassen antidiabetischer Medikamente, die die glukosestimulierte Insulinsekretion bei Ratten und Menschen steigern. Ziele der Arbeit waren es, zu untersuchen, ob die GLP-1 Analoga Exenatid (*E*), Exenatid extended-release (*E e-r*) und der DPP-4 Inhibitor Sitagliptin (*S*) bei Katzen sicher angewendet werden können, das wirksamste Präparat zu identifizieren und die Langzeitwirkung von *E e-r* zu evaluieren. Jeweils drei Katzen erhielten *E* über 5 Tage (0.2-2µg/kg, q12h) subkutan, *E e-r* einmalig (40-400µg/kg) subkutan und *S* über 5 Tage (1-10mg/kg, q24h) peroral. Fünf Tage nach Behandlungsbeginn wurden Mahlzeitenreaktionstests durchgeführt und Flächen unter der Kurve (Areas under the curve; AUC) für Glukose, Insulin und Glukagon kalkuliert. Die Langzeitwirkung von *E e-r* wurde bei drei Katzen während 5 Wochen (100 und 200µg/kg, q7d) untersucht. Verglichen mit den unbehandelten Katzen steigerte *E* die Insulin AUC um 224%, 258%, 331% und 93%, *E e-r* um 127%, 169%, 178% und 95%, und *S* um 32%, 69%, 62%, und 43%. Die AUC von Glukose und Glukagon wurden durch die Medikamente nicht beeinflusst. *E e-r* stimulierte die Insulinsekretion in der Dosierung von 200µg/kg ausgeprägter als mit 100µg/kg. Die drei untersuchten Medikamente können bei Katzen sicher angewendet werden und führen zu einer Steigerung der Insulinsekretion. Die Inkretin-Therapie könnte eine neue Behandlungsoption für Katzen mit Diabetes mellitus darstellen.

Schlüsselwörter: Inkretine, Katzen, Diabetes mellitus, Exenatid, Sitagliptin

Effects of the glucagon-like peptide-1 (GLP-1) analogues exenatide, exenatide extended-release, and of the dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin on glucose metabolism in healthy cats

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Short title: exenatide and sitagliptin in healthy cats.

Abstract

Incretin analogues and inhibitors of the breakdown of endogenous incretins are novel classes of antidiabetic drugs that increase β -cell proliferation and glucose-stimulated insulin secretion in rodents and humans. Here, we tested whether the glucagon-like peptide 1 (GLP-1) analogue exenatide, exenatide extended-release and the dipeptidylpeptidase-4 (DPP-4) inhibitor sitagliptin can be safely used in cats, to identify the most effective drug and to test the effects of prolonged exenatide extended-release administration. Exenatide was given to three cats at 0.2-2 μ g/kg, q12h, subcutaneously, for 5 days. Exenatide extended-release was given to three cats at 40-400 μ g/kg, subcutaneously, once. Sitagliptin was given to three cats at 1-10mg/kg, q24h, orally, for 5 days. Five days after initiation of treatment, meal response tests (MRT) were performed and glucose, insulin and glucagon areas under the curve (AUC) were calculated. Prolonged exenatide extended-release treatment was tested in three cats each at 100 and 200 μ g/kg, q7d, for 5 weeks, followed by MRT. Compared to treatment-naïve cats, exenatide increased insulin AUC by 224%, 258%, 331% and 93%, respectively, exenatide extended-release by 127, 169, 178 and 95%, respectively, and sitagliptin by 32%, 69%, 62%, and 43%, respectively. Glucose and glucagon AUC were unaffected by drug or dose. Repeated administrations of exenatide extended-release at 200 μ g/kg induced a more pronounced enhancement of insulin secretion than at 100 μ g/kg. The three tested drugs are safe to use in cats and enhance insulin secretion. Administration of incretin analogues and inhibitors of incretin breakdown may be beneficial in cats with diabetes mellitus.

Key Words: incretins, cats, diabetes mellitus, exenatide, sitagliptin.

Abbreviations: MRT, meal response test; AUC, area under the curve.

1. Introduction

Incretins are gastrointestinal hormones released during food intake that increase insulin secretion from β -cells of the pancreas. The two well-described incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) share most of their beneficial effects on β -cells such as stimulation of insulin biosynthesis, stimulation of β -cell proliferation, enhanced β -cell survival and suppression of β -cell apoptosis (Farilla et al., 2003; Li et al., 2003). GLP-1 additionally has a suppressive effect on glucagon secretion from pancreatic α -cells as well as various extrapancreatic effects including the slowing of gastric emptying and of gastrointestinal motility, induction of satiation, weight loss, and possibly also cardiovascular protection (Treiman, 2010; Irwin, 2009; Drucker and Nauck, 2006).

Biologically active GLP-1 and GIP have short half-lives (2 minutes and 7 minutes, respectively) and are rapidly degraded by the enzyme dipeptidylpeptidase-4 (DPP-4) (Baggio and Drucker, 2004; Deacon et al., 2000).

Recently, novel classes of anti-diabetic drugs have been developed that take advantage of the glucoregulatory actions of incretins. The incretin-based therapeutics comprise direct GLP-1 receptor agonists, such as exenatide and liraglutide, that are also known as incretin mimetics, and DPP-4-inhibitors, such as sitagliptin, that reduce the degradation of endogenous GLP-1, the so called incretin enhancers. Different formulations of the GLP-1 receptor agonist exenatide exist, requiring either a twice daily (short-acting formulation, e.g. exenatide) or once weekly application (long-acting formulation, e.g. exenatide extended-release). Both drug classes of incretin mimetics and enhancers have been shown to increase β -cell proliferation and glucose-stimulated insulin secretion in rodents and humans, and are successfully used in the treatment of human type 2 diabetes (Blevins et al, 2011; Madsbad et al., 2011; Nauck, 2011; Drucker, 2009; Amori et al., 2007).

Because it is assumed that the pathogenesis of diabetes in most cats is similar to human type 2 diabetes, it can be hypothesized that incretin-based agents could also represent an interesting

therapeutic option in cats (Reusch and Padrutt, 2013). There is only limited data available on the incretin system and the use of incretins in cats. The existence of a physiological incretin effect has been demonstrated recently by showing that a larger amount of glucose was tolerated without development of hyperglycaemia when given orally and therefore passing through the gastrointestinal tract as compared to direct intravenous infusion of glucose. However, the glucose-dependent insulinitropic effect appeared to be much lower than that reported in other species (Gilor et al., 2011a; Gilor et al., 2011b). Further investigation of the incretin system in cats showed a rapid increase of insulin and GLP-1 concentrations after the administration of oral glucose (Hoenig et al., 2011). To the authors' knowledge, only the uses of the GLP-1 receptor agonist exenatide and of the DPP-4 inhibitor NVP-DPP728 have been evaluated in cats prior to this study. It had been shown that exenatide, given once at 1 µg/kg, has a glucose-dependent stimulatory effect on insulin secretion and a pronounced effect on insulin secretion during normoglycemia in cats (Gilor et al., 2011a). The DPP-4 inhibitor NVP-DPP728 showed significant effects to lower plasma glucagon and to enhance insulin secretion (Furrer et al., 2010).

To assess and compare the safety and efficacy of the two short- or long-acting GLP-1 receptor agonists exenatide (Byetta, Amylin Pharmaceuticals Inc., San Diego, CA) and exenatide extended-release (Bydureon, Amylin Pharmaceuticals Inc., San Diego, CA) and of the DPP-4 inhibitor sitagliptin (Januvia, Merck Sharp & Dohme Corp., Whitehouse Station, NJ), and to identify their most effective doses, a dose-escalation study was conducted in healthy cats. In addition, the effect of a prolonged administration of exenatide extended-release was investigated.

2. Materials and methods

2.1. Animals

Two groups of healthy domestic shorthair cats were used. The first group, consisting of nine male cats with a median age of 30 months (range: 24–30) and a median body weight of 5.6 kg (range: 4.8–5.8) was used for the dose-escalation study with exenatide, exenatide extended-release and sitagliptin. The second group, consisting of six male cats with a median age of 13 months (range: 13–15) and a median body weight of 5.1 kg (range: 4.9–5.6) was used to test the prolonged administration of exenatide extended-release. The cats were group-housed, had free access to water and were fed twice daily at 08:00 am and 06:00 pm with a commercial dry food diet (Adult Indoor, Hill's Pet Nutrition Inc., Topeka, KS). Environmental enrichment and social contact were provided on a daily basis. The experiment was approved by the Veterinary Office of the Canton Zurich, Switzerland (nr 122/2011).

Well-being and safety of treatments were assessed in all cats twice daily throughout the experiment and for an additional week thereafter by physical examination and measurement of body weight, by monitoring appetite, food and water intake, and by recording possible clinical side effects. With regard to the latter, particular attention was paid to those of the gastrointestinal tract, including vomiting, soft stools and diarrhoea, because similar side effects are frequently reported in humans (Holst, 2012; Campbell, 2011). Quality of stools was determined according to the Purina Fecal Scoring System (www.purinaveterinarydiets.com). Because DPP-4 inhibitors may predispose to cystitis and nasopharyngitis in humans (Amori et al., 2007) clinical signs of the urinary tract, such as stranguria and pollakiuria, and of the upper respiratory tract, such as sneezing and nasal discharge, were also noted in individual medical records for each cat.

2.2. Blood sampling

One week before starting the experiment, a 5 ml blood sample was obtained from the jugular vein in awake animals. Blood samples were collected to perform haematological and serum biochemical profiles and to verify the health status of the cats. During the experiment, blood samples were taken via a jugular catheter to minimize stress reactions of the cats. Jugular catheters were placed by Seldinger technique under general anaesthesia on each day of meal response testing (Seldinger 1953). The animals were fasted overnight (from 06:00 pm on the evening prior to MRT) to ensure a minimal risk of aspiration of gastric contents during anesthesia for jugular catheter placement, according to AAHA Anesthesia Guidelines for Dogs and Cats (Bednarski et al., 2011). To insert the catheter, sedation was obtained with intramuscular butorphanol (Morphasol®-4, Dr. E. Graeb AG, Bern, Switzerland) at 2 mg/kg and the α_2 -adrenoceptor agonist medetomidine (Domitor®, ProVet AG, Lyssach, Switzerland) at 5 μ g/kg, and general anaesthesia was maintained with intravenous propofol (Propofol 1% MTC Fresenius, Fresenius Kabi AG, Bad Homburg, Germany) at 6-8 mg/kg. After implantation of the jugular catheters, atipamezol, a potent, selective and specific antagonist of both centrally and peripherally located α_2 -adrenoceptors was administered at 5 μ g/kg (Antisedan®, ProVet AG, Lyssach, Switzerland) to reverse the effects of medetomidine and the cats regained full consciousness within 5-10 minutes.

Blood samples were collected during each meal response test (MRT) and were used to measure plasma concentrations of insulin and glucagon. After collection, samples were immediately transported to the laboratory on ice, cold-centrifuged and the EDTA plasma was stored at -80 °C until later analysis. Insulin was measured with the Mercodia ELISA # 10-1233-01 (Mercodia AB, Uppsala, Sweden) and glucagon with the rat-glucagon ELISA kit Wako # 297-57101 (Wako Chemicals GmbH, Neuss, Germany), previously validated for use in cats by our laboratory. Capillary blood glucose was measured with the portable glucose meter AlphaTRAK (Abbott Laboratories, Abbott Park, IL) specifically designed for dogs and

cats, using samples collected from the inner pinna of the ear. The device has been shown to accurately measure blood glucose in cats (Zini et al., 2009).

2.3. Meal response test

The MRT was performed prior to initiation of treatment and on day 5 of each incretin treatment period. The MRT served to assess α - and β -cell function in cats (Furrer et al., 2010). One hour after regaining full consciousness following the placement of the jugular catheters, 2 ml basal blood samples were taken and the cats were offered a test meal (Adult Indoor, Hill's Pet Nutrition Inc., Topeka, KS) comprising 50% of their average daily energy requirement (25 g, approximately 130 kcal). The test meals were rapidly ingested (in 1-2 minutes). Subsequently, 2 ml blood samples were taken from the jugular catheters at 15, 30, 60, 120 and 300 minutes after food intake.

2.4. Dose-escalation protocol

Because no data were available regarding exenatide, exenatide extended-release and sitagliptin administration in cats, a dose-escalation experiment was conducted for each drug to identify the occurrence of possible clinical adverse reactions and to assess their effect on insulin and glucagon secretion. After performing a basal MRT prior to treatment, which was followed by a 2-week intermission without any medication, treatment was started in all three drug groups. Exenatide was given subcutaneously to each of three cats at 0.2, 0.5, 1 and 2 $\mu\text{g}/\text{kg}$ for five consecutive days, twice daily (at 8:00 am and 8:00 pm). Exenatide extended-release was given subcutaneously to each of three cats at 40, 100, 200 and 400 $\mu\text{g}/\text{kg}$ with single injections (at 8:00 am on day 1). Sitagliptin was given orally to each of three cats at 1, 3, 5 and 10 mg/kg for five consecutive days, once daily (at 8:00 am). To prevent accumulation of the drugs, every new dose was anticipated by a 2-week washout period. On day 5 of each treatment dose of exenatide or sitagliptin, or on day 5 following each injection

of exenatide extended-release, MRT were performed starting at 11:00 am in all cats. To assess the effects of the drugs, AUC for glucose, insulin and glucagon were compared to values before drug administration.

2.5. Prolonged administration of exenatide extended-release

Evidence from human medicine showed that a steady state plasma level of exenatide-extended release may only be reached after 6-8 weeks of treatment (Iwamoto et al., 2009; Kim et al., 2007). Therefore prolonged administration of exenatide extended-release was also performed in the present study in cats. Using the two safest and most effective drug doses estimated from the dose-escalation experiment, exenatide extended-release was injected subcutaneously to three cats at the dose of 100 µg/kg and to another three cats at the dose of 200 µg/kg, every seven days, for five consecutive weeks. The MRT was performed five days after the last exenatide extended-release administration and AUC for glucose, insulin and glucagon were calculated. Values prior to treatment were compared to the values obtained on day five (11:00 am) after the last administration of exenatide extended-release.

3. Results

Drug safety and side effects

In the nine cats used for the dose-escalation study, mild and self-limiting diarrhoea and vomiting occurred in six of nine cats (Table 1). Diarrhoea (scores 5 and 6 of the Purina Fecal Scoring System (score 1 to 7)) was documented in six cats (2 with exenatide, 2 with exenatide extended-release and 2 with sitagliptin), for a median duration of four days (range: 1-5); vomiting was documented in two cats with diarrhoea (1 with exenatide, 1 with exenatide extended-release), for one and six days, respectively. In two cats the side effects developed during the first day of treatment, and in one cat after the second day of treatment. In three cats side effects first occurred with a new dose, on the first day or on the second to fifth day of

treatment. In addition, in four of the six cats side effects were also observed during the 2-week washout period. Of note, in two cats side effects were documented once during the experiment and in four cats they occurred several times (median: 5 times, range: 5–7). The gastrointestinal adverse reactions were self-limiting; none of the cats needed therapeutic intervention and application of the drugs did not have to be discontinued in any of them. Other side effects were not documented. General well-being as well as food and water intake were not impaired at any time. In the second group of six cats used for the prolonged administration of exenatide extended-release, side effects did not occur. Reactions at the injection-site were not observed in any of the cats receiving either exenatide or exenatide extended-release.

Dose-escalation protocol

Average glucose AUC after the administration of exenatide, exenatide extended-release and sitagliptin were similar to corresponding glucose AUC obtained prior to treatment, irrespective of the dose (Tables 2a, 2b, 2c). Hypoglycemia did not occur with any drug. All three drugs tested during the dose-escalation protocol enhanced meal-dependent insulin secretion, based on MRT. The 4 incremental doses of exenatide increased average insulin AUC by 224%, 258%, 331% and 93%, respectively, compared to insulin AUC in treatment naïve cats (Table 3a). Exenatide extended-release doses increased average insulin AUC by 127%, 169%, 178%, 95%, respectively, and sitagliptin increased average insulin AUC by 32%, 69%, 62%, 43%, respectively (Tables 3b, 3c).

Exenatide increased and decreased glucagon AUC, whereas exenatide extended-release and sitagliptin decreased glucagon AUC, based on MRT. The three lower doses of exenatide yielded an average increase of 14%, 35%, 46%, respectively, while the highest dose of exenatide decreased glucagon AUC by 14% compared to glucagon AUC obtained in treatment-naïve cats (Table 4a). Exenatide extended-release decreased average glucagon AUC

by 15%, 8%, 1% and 16%, respectively, and sitagliptin decreased average glucagon AUC by 34%, 29%, 16% and 22%, respectively (Tables 4b, 4c).

Prolonged administration of exenatide long-acting

Average glucose AUC after the prolonged administration of exenatide extended-release was similar to glucose AUC obtained prior to treatment, with either dose (Table 5a).

Hypoglycemia was not documented.

Based on MRT, the prolonged administration of exenatide extended-release 100 µg/kg/week for 5 weeks decreased average insulin AUC by 8% compared to treatment-naïve cats (Table 5b); of note, although insulin AUC was reduced, the insulin AUC from 0 to 120 minutes in treated cats was 40% higher than in untreated cats.. Exenatide extended-release at the dose of 200 µg/kg/week for 5 weeks increased average insulin AUC during MRT by 15% (Table 5b) and the insulin AUC from 0 to 120 minutes in treated cats was 116% higher than in untreated cats.

Based on MRT, the prolonged administration of exenatide extended-release 100 µg/kg/week for 5 weeks increased average glucagon AUC by 253 % in comparison to treatment-naïve cats. Exenatide extended-release at the dose of 200 µg/kg/week for 5 weeks increased average glucagon AUC by 3% (Table 5c).

4. Discussion

The results of the dose-escalation protocol showed that exenatide, exenatide extended-release and sitagliptin were all able to enhance insulin secretion in healthy cats. The GLP-1 agonists exenatide and exenatide extended-release showed more pronounced effects than the DPP-4 inhibitor sitagliptin which is consistent with findings in humans, where GLP-1 agonists are superior in achieving good glycaemic control (Karagiannis et al., 2012; Nauck, 2011). GLP-1 leads to an increase of insulin secretion in humans due to direct effects on pancreatic β -cells, including a stimulation of insulin biosynthesis and of β -cell proliferation, as well as enhanced β -cell survival and resistance to apoptosis (Farilla et al., 2003; Li et al., 2003). Because most diabetic cats suffer from a disease that shares many similarities to human type 2 diabetes (Reusch and Padruitt, 2013), the enhanced insulin secretion achieved in healthy cats may suggest that exenatide, exenatide extended-release and sitagliptin could be beneficial also in diabetic cats, possibly improving β -cell function or viability via one or more of the mechanisms demonstrated in humans.

Prolonged administration of exenatide extended-release enhanced postprandial insulin secretion more than in untreated cats, at both 100 and 200 $\mu\text{g/kg/week}$. The effects on insulin secretion were more pronounced at the higher dose, where the meal-induced enhancement of insulin concentration of the AUC from 0 to 120 minutes was 116% higher after the 5-week application of the drug than in treatment naïve cats. The comparison of AUCs from 0 to 120 minutes of untreated vs. treated cats (40% increase of AUC for 100 $\mu\text{g/kg}$ and 116% increase for 200 $\mu\text{g/kg}$) showed a pronounced post prandial enhancement of insulin secretion for both doses, whereas the total AUC of insulin was less affected by exenatide extended-release, with a mild increase at 200 $\mu\text{g/kg}$ and even a slight decrease with 100 $\mu\text{g/kg}$. The reason for the mild effect on insulin AUC is probably due to the fast insulin drop observed after the postprandial insulin peak in treated cats. This drop in insulin secretion after the time period of 120 minutes could possibly be explained by counterregulatory mechanisms against

hypoglycaemia in healthy cats. In dogs, counterregulatory elevations of glucagon, cortisol and lactate have been observed after portal administration of GLP-1 (Ionut et al., 2005), and studies in humans show that in the presence of exenatide there are strongly preserved counterregulatory mechanisms during hypoglycemia (Degn et al., 2004). Although during the present study hypoglycemia did not occur, similar counterregulatory effects might have prevented impending substantial drops in blood glucose in the healthy cats.

A tachyphylactic effect of the tested drugs during the dose-escalation protocol and the prolonged administration of exenatide extended-release seems rather unlikely. Also after chronic administration of GLP-1 receptor agonists, despite sustained elevations in the circulating levels of the drug, no evidence for tachyphylaxis or clinically significant receptor desensitization has been observed in human clinical studies (Nauck et al., 1996, Hargrove et al., 1995). Exendin 4 has been found to be more potent than native GLP-1 in producing GLP-1 receptor desensitization *in vitro*, but chronic exposure to exendin 4 in normal or transgenic mice was not associated with significant downregulation of GLP-1 receptor-dependent responses coupled to glucose homeostasis (Baggio et al., 2004).

The strong effects on insulin peak secretion and the absence of any side effects as well as the convenient therapy regimen of a once weekly injection may make the prolonged administration of exenatide extended-release a more favourable option over exenatide and sitagliptin in diabetic cats.

Besides the effect of GLP-1 on insulin, incretins also decrease glucagon secretion from pancreatic α -cells (Drucker and Nauck, 2006). In diabetic humans, GLP-1 agonists as well as DPP-4 inhibitors reduce glucagon secretion. In a previous study in healthy cats, the administration of the DPP-4 inhibitor (NVP-DPP728) yielded a significant reduction of plasma glucagon concentrations after a MRT (Furrer et al., 2010). Consistent with these findings, results of the present dose-escalation protocol showed a decrease of glucagon secretion with sitagliptin and exenatide extended-release. Differently, with exenatide a dose-

dependent increase of glucagon followed by a decrease was observed. The reason for this result is unclear. It is known that in the case of hypoglycemia, counterregulatory secretion of glucagon is preserved even with pharmacological concentrations of GLP-1 (Drucker and Nauck, 2006). Although the measured glucose values during MRT did not drop, it might be that the observed increase of glucagon secretion was part of the physiologic compensation against hypoglycaemia in healthy cats. Furthermore, the decrease of glucagon demonstrated with the highest exenatide dose (i.e., 2 µg/kg) may be explained by the fact that the same dose lead to the lowest increase of insulin concentration, perhaps reducing the counterregulatory effect on glucagon.

Similar to most doses of exenatide, prolonged administration of exenatide extended-release increased glucagon secretion, in particular at the lower dose (100 µg/kg). The reason for the increased glucagon, which contrasts the effect of exenatide extended-release given for a short period during the dose-escalation protocol, is uncertain. At 100 µg/kg of exenatide extended-release, the cats showed lower insulin secretion, making the above hypothesis of counterregulation provided for the highest dose of exenatide unlikely. The present findings of this study cannot provide evidence of a suppressive effect of exenatide, exenatide extended-release, and sitagliptin on glucagon concentrations. Further studies are required to assess the effects of incretin mimetics and enhancers on glucagon.

The results of glucose AUC obtained after treatment did not differ from those achieved prior to treatment with any of the drugs tested, and no lowering effect on fasting glucose was observed. Since only healthy animals were used for this study, counteracting hormones, such as glucagon but also norepinephrine (Rand et al., 2008), might have played a substantial role in preventing hypoglycaemia or even slight drops in blood glucose concentrations. A recent study in healthy rats showed an unexpected rise of glucose levels after acute administration of exenatide caused by stimulation of the sympathetic nervous system (Perez-Tilve et al., 2010).

A similar effect might be present also in healthy cats, possibly explaining the lack of glucose lowering effects of incretins under our test conditions, in healthy normoglycemic cats.

As an additional benefit to improved glycaemic control, the GLP-1 agonists exenatide and exenatide extended-release are associated with weight loss in diabetic patients, whereas DPP-4 inhibitors seem to be weight neutral (Blevins et al., 2011; Madsbad et al., 2011; Drucker et al., 2008.). Body weight of all the cats was assessed on a weekly basis and remained stable during the study period. It cannot be excluded that longer duration of treatment might also have caused weight loss in cats.

The results of this study show that exenatide, exenatide extended-release and sitagliptin can all cause gastrointestinal side effects in cats. In particular, cats enrolled in the dose-escalation protocol showed mild and self-limiting diarrhoea and vomiting that were independent from the drug and dose given. Differently, exenatide extended release administered to cats over a period of 5 weeks did not appear to cause gastrointestinal side effects.

Exenatide is generally well tolerated in humans, although commonly reported side effects include mild gastrointestinal symptoms like nausea, vomiting and diarrhoea, which usually resolve after 4 to 8 weeks of treatment (Holst, 2012; Campbell, 2011). Gastrointestinal symptoms are dose-dependent and gradual dose increase can be used to lessen symptoms (Fineman et al., 2004). In the present study, clinical signs with exenatide were similar to humans, but their occurrence did not appear to be dose-dependent, at least within the dose range tested here, possibly suggesting a species-specific difference.

The frequency of gastrointestinal side effects with exenatide extended-release is lower than with exenatide, but the former commonly causes injection-site reactions in humans (Blevins et al., 2011; Madsbad et al., 2011; Drucker et al., 2008). In cats, side effects with exenatide extended-release were few in the dose-escalation protocol and none during the prolonged administration, similar to humans. Obvious reactions at the injection site were not documented, suggesting that exenatide extended-release would be very safe if used in diabetic

cats. DPP-4 inhibitors like sitagliptin are known to cause less gastrointestinal side effects than GLP-1 agonists, but are associated with an increased risk of cystitis, nasopharyngitis and headache (Amori et al., 2007). None of the cats of the study showed respiratory signs or urinary tract disease.

Limitations of the study that need to be mentioned are the small number of cats included that prevented inferential statistical analysis to be performed, as well as the lack of blood samples collected after treatment to identify possible side effects on haematological and serum biochemical profiles. Because of the intensive study protocol and multiple blood sampling of considerable blood volumes, the allowed number of research animals was limited to 9 by the veterinary office. To prevent anemia, on the grounds of animal welfare considerations as well as to preclude potential negative effects of anemia on the test results, the study period was limited to the described 4 incremental doses of each drug.

In conclusion, exenatide, exenatide extended-release and sitagliptin can be safely used and are able to enhance insulin secretion in healthy cats. The use of incretin-based therapy may represent a novel treatment option for management of diabetic cats, and warrants further studies in a larger population of healthy cats, as well as diabetic cats.

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Figure captions

Table 1. Overview of gastrointestinal side effects due to exenatide, exenatide extended-release and sitagliptin. D= diarrhoea, V= vomitus.

Table 2. Glucose AUC concentrations with exenatide (a), exenatide extended-release (b) and sitagliptin (c) at incremental doses. The areas are expressed in (mmol/L) x minutes.

Table 3. Insulin AUC concentrations with exenatide (a), exenatide extended-release (b) and sitagliptin (c) at incremental doses. The areas are expressed in (pmol/L) x minutes.

Table 4. Glucagon AUC concentrations with exenatide (a), exenatide extended-release (b) and sitagliptin (c) at incremental doses. The areas are expressed in (ng/L) x minutes.

Table 5. Glucose (a), insulin (b) and glucagon (c) AUC concentrations with exenatide extended-release at 100 µg/kg/week and 200 µg/kg/week for five weeks. The areas are expressed in (mmol/L) x minutes.

Table 1

	Cats with clinical signs	D	V	Onset of clinical signs per cat	Total frequency of clinical signs per cat	Cats with clinical signs during washout
Exenatide	2	2	1	- Dose 1, day 1 - Dose 3, day 1	- 6 times - once	1
Exenatide extended-release	2	2	1	- Dose 1, day 2 - Dose 2, day 1	- 5 times - 7 times	2
Sitagliptin	2	2	-	- Dose 1, day 1 - Dose 4, day 3	- 5 times - once	1

Table 2a

Exenatide Dose	Prior to treatment	0.2 µg/kg q12h	0.5 µg/kg q12h	1 µg/kg q12h	2 µg/kg q12h
Cat A	1459	1595	1522	1601	1324
Cat B	1454	1594	1597	1960	1854
Cat C	1656	1519	1423	1516	1271

Table 2b

Exenatide extended- release Dose	Prior to treatment	40 µg/kg	100 µg/kg	200 µg/kg	400 µg/kg
Cat D	1459	1964	2033	6164	1878
Cat E	1459	1970	1634	1726	1653
Cat F	1913	1709	1498	1545	1634

Table 2c

Sitagliptin Dose	Prior to treatment	1 mg/kg q24h	3 mg/kg q24h	5 mg/kg q24h	10 mg/kg q24h
Cat G	1573	1763	1539	1379	1660
Cat H	1452	1508	1311	1379	1315
Cat I	1222	1276	1381	1364	1491

Table 3a

Exenatide Dose	Prior to treatment	0.2 µg/kg q12h	0.5 µg/kg q12h	1 µg/kg q12h	2 µg/kg q12h
Cat A	4183	30348	23582	28408	10244
Cat B	5529	19649	29192	26921	13241
Cat C	4401	9281	12719	23555	11853

Table 3b

Exenatide extended- release Dose	Prior to treatment	40 µg/kg	100 µg/kg	200 µg/kg	400 µg/kg
Cat D	3305	10406	24410	24768	21492
Cat E	10409	16833	11460	16485	12700
Cat F	8576	23229	23983	20606	9205

Table 3c

Sitagliptin Dose	Prior to treatment	1 mg/kg q24h	3 mg/kg q24h	5 mg/kg q24h	10 mg/kg q24h
Cat G	4183	30348	23582	28408	10244
Cat H	5529	19649	29192	26921	13241
Cat I	4401	9281	12719	23555	11853

Table 4a

Exenatide Dose	Prior to treatment	0.2 µg/kg q12h	0.5 µg/kg q12h	1 µg/kg q12h	2 µg/kg q12h
Cat A	46997	36082	37799	63697	30441
Cat B	75334	53183	69441	105421	44998
Cat C	52298	111341	129375	87462	75687

Table 4b

Exenatide extended- release Dose	Prior to treatment	40 µg/kg	100 µg/kg	200 µg/kg	400 µg/kg
Cat D	47175	61473	48565	55003	36373
Cat E	38924	27702	39594	49246	53491
Cat F	94955	64379	78101	74924	61893

Table 4c

Sitagliptin Dose	Prior to treatment	1 mg/kg q24h	3 mg/kg q24h	5 mg/kg q24h	10 mg/kg q24h
Cat G	64787	38913	40615	56009	42378
Cat H	59742	35010	35562	41004	34756
Cat I	38372	31242	39136	40671	49658

Table 5a

Exenatide extended- release Dose	Prior to treatment	100 µg/kg q7d for 5 weeks	Prior to treatment	200 µg/kg q7d for 5 weeks
Cat D	1208	1202	1347	1238
Cat E	1188	1200	1378	1264
Cat F	1201	1255	1254	1206

Table 5b

Exenatide extended- release Dose	Prior to treatment	100 µg/kg q7d for 5 weeks	Prior to treatment	200 µg/kg q7d for 5 weeks
Cat D	11968	6003	18691	11443
Cat E	8646	15065	11831	17586
Cat F	13193	9975	7591	14891

Table 5c

Exenatide extended- release Dose	Prior to treatment	100 µg/kg q7d for 5 weeks	Prior to treatment	200 µg/kg q7d for 5 weeks
Cat D	136516	170301	73051	144057
Cat E	45742	641230	157142	89090
Cat F	133196	301457	70719	78143

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